

The Space Life: Microbes on Surfaces and in the Air aboard the International Space Station

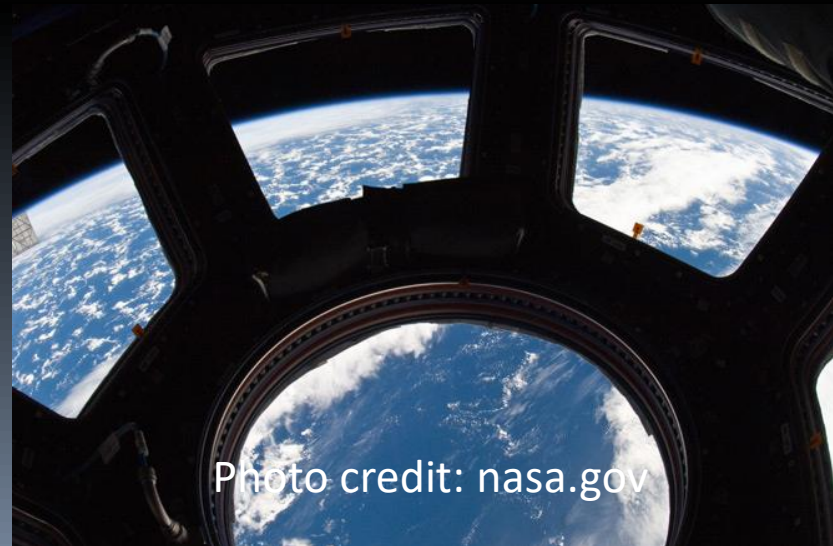
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March 27, 2018



Overview

- International Space Station as a living environment
- Research – microbes on the surfaces and in the air of the ISS
- Conclusions

International Space Station (ISS)

- Launched on November 20th, 1998
- Low Earth Orbit (LEO) – altitude between 330 – 435 km (205 – 270 mi)
- The brightest object on the sky
- Size of a football field
- Orbits Earth at 5 miles per second
- Full crew: 6 people
- Weights ~ 1 mln pounds



Photo credit: nasa.gov

Unique features of the ISS

1. Microgravity
2. Extreme conditions:
 - a) Vacuum
 - b) Atomic oxygen
 - c) Ultraviolet radiation
 - d) Particulate or ionizing radiation
 - e) Plasma
 - f) Temperature extremes

Microorganisms

- Smallest microorganisms that constitute bacteria, archaea, fungi, viruses, protozoa, algae
- Ubiquitous and abundant in the environment
- Have a variety of essential functions
- Very often adapted to specific environmental niches, e.g. those that inhabit the boiling water springs in Yellowstone National Park

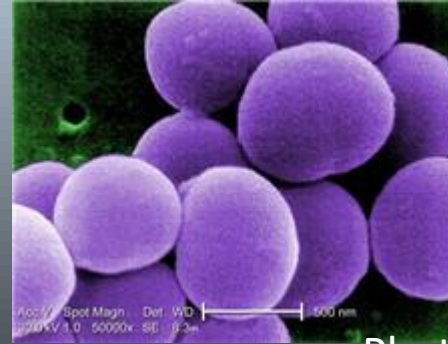


Photo credit:
cdc.gov



Mysterious creatures?

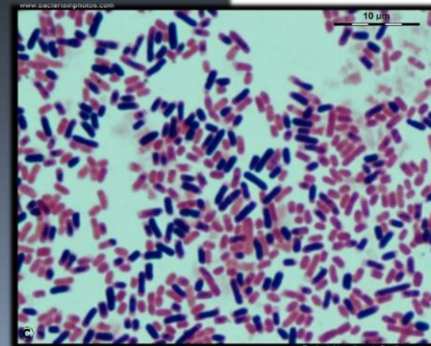
“Most types of microbes remain unknown. It is estimated that we know fewer than 1% of the microbial species on Earth. Yet microbes surround us everywhere - air, water, soil. An average gram of soil contains one billion (1,000,000,000) microbes representing probably several thousand species.” *International Society of Microbial Ecology*

Detection of Microbes – Traditional Methods

1. Enumeration – growth on the Petri dishes plate in solidified medium
2. Identification based on the morphology

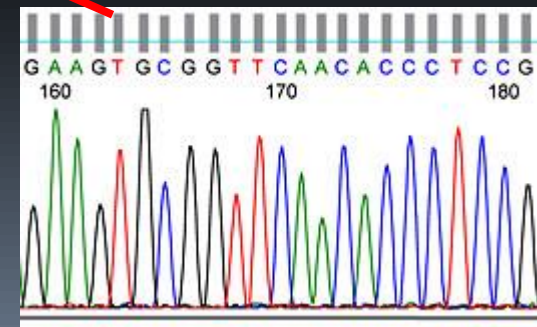
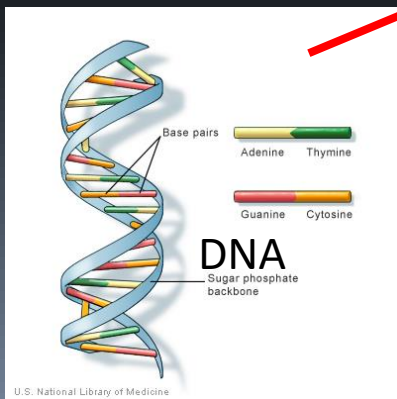
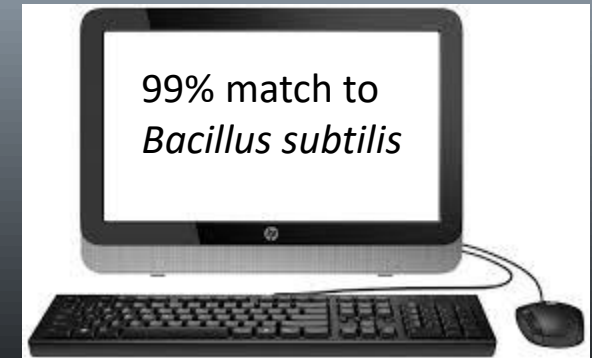
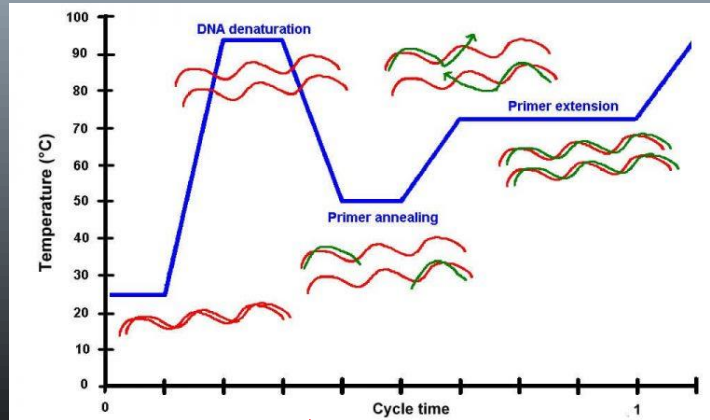


Culture plates

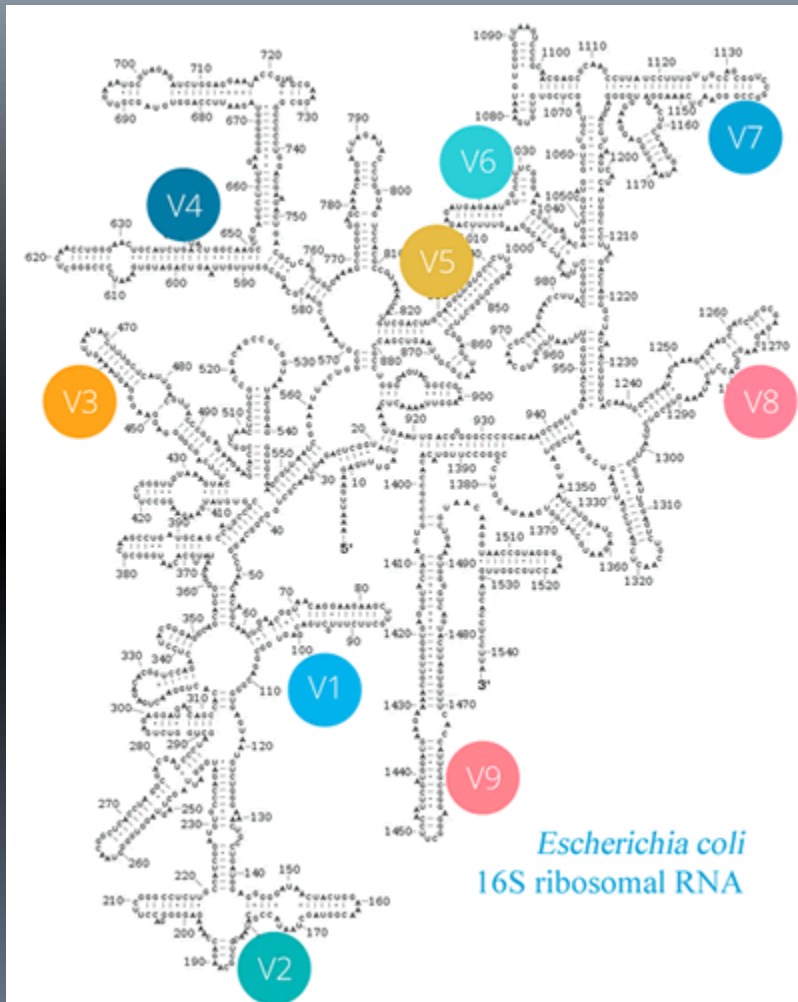


Microscopic techniques

Detection of Microbes – Molecular Methods



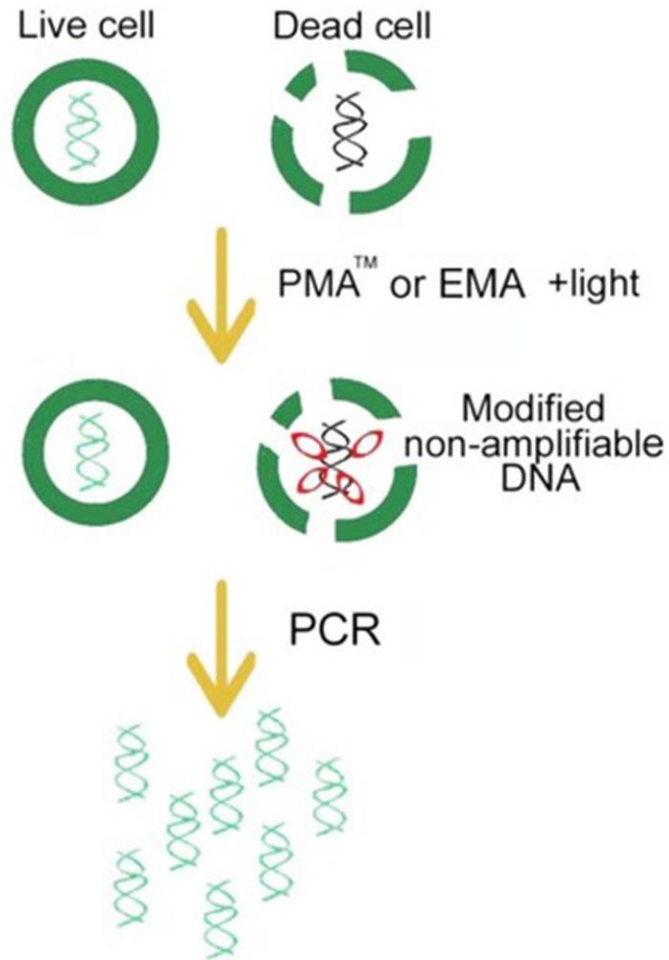
Identification via 16S rRNA



		Variable 1 DNA
Cyanobacteria	<i>Myxosarcina</i> PCC7312	GCTGGCGGTATGCCTAACACATGCAATCGAACGAA--AT-C-----TTCG-----G--ATTT-AGTGGCGGACG
	<i>Pseudanabaena</i> PCC7403	GCTGGCGGTATGCTTAACACATGCAATCGAACGAA--AT-C-----TTCG-----G--ATTT-AGTGGCGGACG
	<i>Synechococcus</i> WH8101	GCTGGCGGCGTGTCTTAACACATGCAATCGAACG-A--AC-C-----TTCG-----G--GTT-AGTGGCGGACG
	<i>Prochlorococcus</i> MIT9312	GCTGGCGGCGTGTCTTAACACATGCAATCGAACG-A--AC-C-----TTCG-----G--GTT-AGTGGCGGACG
	<i>Synechococcus</i> PCC6307	GCTGGCGGCGTGTCTTAACACATGCAATCGAACG-A--GC-C-----TTCG-----G--GCT-AGTGGCGGACG
	<i>Cyanothece</i> PCC7418	GCTGGCGGCGTGTCTTAACACATGCAATCGAACGAA--GC-C-----CTCG-----G--GCTT-AGTGGCGGACG
	<i>Oscillatoria neglecta</i>	GCTGGCGGCGTGTCTTAACACATGCAATCGAACGAA--CC-C-----TTCG-----G--GTT-AGTGGCGGACG
	<i>Leptolyngbya</i> PCC7375	GCTGGCGGCGTGTCTTAACACATGCAATCGAACGAA--CC-T-----TTCG-----G--GTTG-AGTGGCGGACG
	<i>Symploca</i> VP377	GCTGGCGGTATGCTTAACACATGCAATCGAACGCA--CC-T-----TTCG-----G--GTTG-AGTGGCGGACG
	<i>Microcoleus</i> PCC7420	GCTGGCGGTATGCCTAACACATGCAATCGAACGCA--AC-C-----TTCG-----G--GTTG-AGTGGCGGACG
	<i>Synechococcus</i> PCC7002	GCTGGCGGTATGCCTAACACATGCAATCGAACGAA--GC-C-----TTCG-----G--GCTT-AGTGGCGGACG
	<i>Chamaesiphon</i> PCC7430	GCTGGCGGTATGCCTAACACATGCAATCGAACGAA--AC-C-----TTCG-----G--GTTT-AGTGGCGGACG
	<i>Gloeotheca</i> SK40	GCTGGCGGTATGCTTAACACATGCAATCGAACGAA--GT-C-----TTCG-----G--ACTT-AGTGGCGGACG
	<i>Prochlorothrix hollandica</i>	GCTGGCGGCGTGTCTTAACACATGCAATCGAACGAA--GT-C-----TTCG-----G--ACTT-AGTGGCGGACG
	<i>Trichodesmium</i> NIBB1067	GCTGGCGGTATGCTTAACACATGCAATCGAACGAA--CT-C-----TTCG-----G--AGTT-AGTGGCGGACG
	<i>Prochloron</i>	GCTGGCGGTATGCTTAACACATGCAATCGAACGAA--CT-C-----TTCG-----G--AGTT-AGTGGCGGACG
	<i>Synechocystis</i> PCC6803	GCTGGCGGTATGCCTAACACATGCAATCGAACGAA--GTTT-----TTCG-----G--AACTT-AGTGGCGGACG
	<i>Chroococcidiopsis</i> PCC7203	GCTGGCGGTATGCTTAACACATGCAATCGAACGAA--GCTT-----TTCG-----G--AGCTT-AGTGGCGGACG
	<i>Nostoc</i> PCC7120	GCTGGCGGTATGCTTAACACATGCAATCGAACGGT--CT-C-----TTCG-----G--AGAT-AGTGGCGGACG
β -proteobacteria	<i>Leptolyngbya</i> PCC73110	GCTGGCGGTATGCTTAACACATGCAATCGAACGGT--CT-C-----TTCG-----G--AGAT-AGTGGCGGACG
	<i>Spirulina</i> PCC6313	GCTGGCGGTATGCT--AACACATGCAATCGAACGGT--CT-C-----TTCG-----G--AGAT-AGTGGCGGACG
	<i>Chlorogloeopsis</i> PCC7518	GCTGGCGGTATGCT--AACACATGCAATCGAACGGT--CT-G-----GAAA-----C--AGAC-AGTGGCGGACG
	<i>Synechococcus</i> IR11	GCTGGCGGCGTGTCTTAACACATGCAATCGAACGGG--TG-C-----TTCG-----G--CACT-AGTGGCGGACG
	<i>Microcystis</i> PCC7941	GCTGGCGGCGTGTCTTAACACATGCAATCGAACGGG--AATC-----TTCG-----G--ACTCT-AGTGGCGGACG
	<i>Arthrospira</i> PCC8005	GCTGGCGGCGTGTCTTAACACATGCAATCGAACGGG--CT-C-----TTCG-----G--AGCT-AGTGGCGGACG
	<i>Synechococcus</i> PCC6301	GCTGGCGGCGTGTCTTAACACATGCAATCGAACGGG--CT-C-----TTCG-----G--AGCT-AGTGGCGGACG
	<i>Gloeobacter</i> PCC7421	GCTGGCGGCGTGTCTTAACACATGCAATCGAACGGG--AT-C-----TTCG-----G--ATCG-AGTGGCGGACG
	Strain CMEE 5410	GCTGGCGGTATGCTTAACACATGCAATCGAACGGGAGCA--CGAG--GAGCTTGCTC--CTTGG--TGCGAGTGGCGGACG
	<i>A. marina</i> MBIC-11017	GCTGGCGGTATGCTTAACACATGCAATCGAACGGGAGCA--CGAG--GAGCTTGCTC--CTTGG--TGCGAGTGGCGGACG
γ -proteobacteria	<i>Bordetella</i> 61717	GCTGGCGGTATGCTTAACACATGCAATCGAACGGGAGCA--CGAG--GAGCTTGCTC--CTTGG--TGCGAGTGGCGGACG
	<i>Alcaligenes</i> LMG-5906	GCTAGCGGGATGCTTTACACATGCAATCGAACGGGAGCA--CGAA--GAGCTTGCTC--TTTGG--TGCGAGTGGCGGACG
	<i>Alcaligenes</i> defragrans	GCTAGCGGGATGCTTTACACATGCAATCGAACGGGAGCA--CGAGAGAGCTTGCTCCTTTGG--TGCGAGTGGCGGACG
	<i>Alcaligenes</i> faecalis	GCTAGCGGGATGCTTTACACATGCAATCGAACGGGAGCA--CG--G--GAGCTTGCTC--C--TGG--TGCGAGTGGCGGACG
	<i>Nitrobacter</i> cryotolerans	GCTAGCGGGATGCTTTACACATGCAATCGAACGGGAGCA--CG--G--GAGCTTGCTC--C--TGG--TGCGAGTGGCGGACG
	<i>Achromobacter</i> xylosoxidans	GCTAGCGGGATGCTTTACACATGCAATCGAACGGGAGCA--CG--G--GAGCTTGCTC--C--TGG--TGCGAGTGGCGGACG
	<i>Bordetella pertussis</i>	GCTAGCGGGATGCTTTACACATGCAATCGAACGGGAGCA--CG--G--GAGCTTGCTC--C--TGG--TGCGAGTGGCGGACG
	<i>Escherichia coli</i>	GCTGGCGGAGGCTTAACACATGCAATCGAACGGGAGCA--AAGCTTGCTC--CTTTGCTGACAGTGGCGGACG
	<i>Rhodobacter</i> sphaeroides	GCTGGCGGAGGCTTAACACATGCAATCGAACGGGAGCA--GT-C-----TTCG-----G--ACTT-AGCGCGGAGACG
		*** **

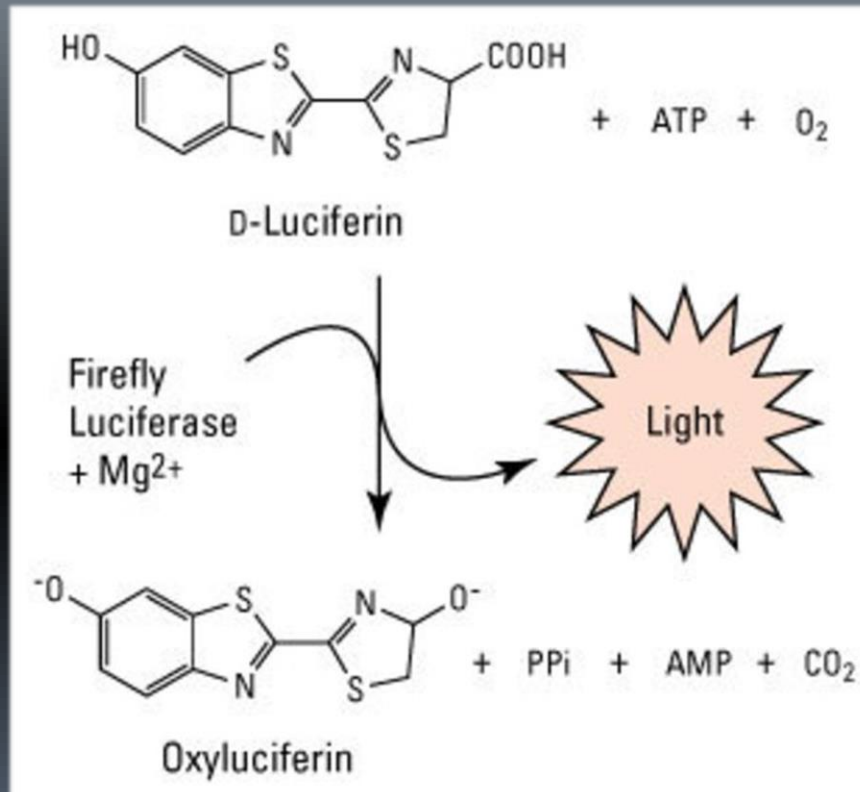
Discovery of a free-living chlorophyll *d*-producing cyanobacterium with a hybrid proteobacterial/cyanobacterial small-subunit rRNA gene. Miller et al. (2005). PNAS

Propidium Monoazide Treatment to Distinguish between Dead and Live Cells



www.biotium.com

Bioluminescence Assay – Metabolically Active Cells



<https://nhmu.utah.edu>

Thermofisher.com

Study of Air and Surfaces

Clean room

- Multi-personnel
- Gowning (“bunny suit”, masks, gloves)
- Gravity
- Radiation
- Activities limited to work (assembly of spacecrafts and instruments)



Photo credit: nasa.gov

ISS

- Crew of 6 people
- Casual clothes; short-sleeved shirts, shorts
- Microgravity
- Elevated radiation
- Work and everyday activities (working, eating, sleeping, exercising, and etc.)

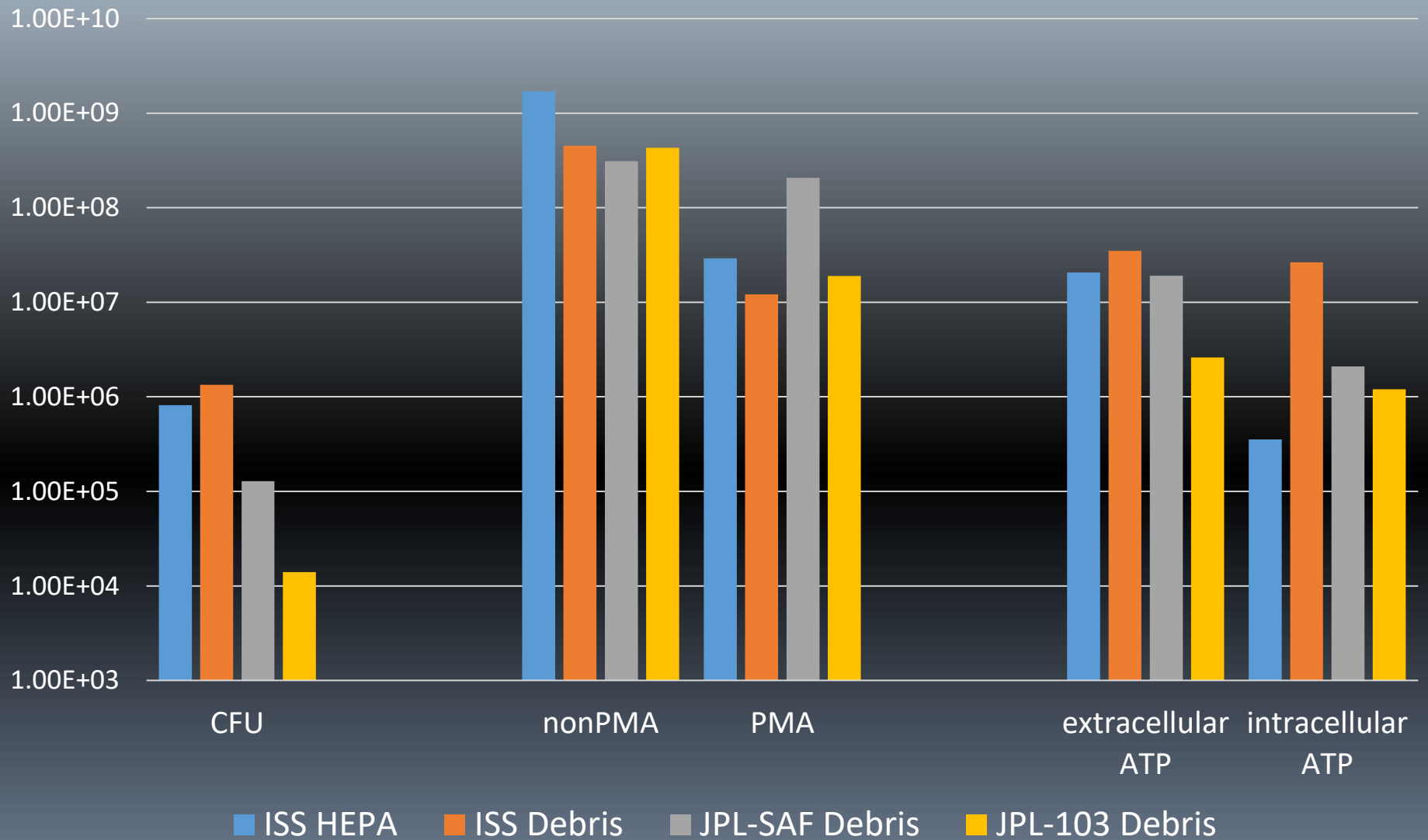


Detailed Sample Characteristics

Sample name	Location	Source	Type	Specifications	Duration	Model	Mission activities
ISS HEPA	ISS Node 2	HEPA filter element	Air	HEPA rated, retains 99.97% particles >0.3 μm ; 20-mesh inlet screen has 841 μm sieve openings	40 months	Part no. SV810010-1, Serial no. 0049; HEPA media supplied by Flanders Filters, Inc.; Nomex inlet screen	Returned aboard STS-134/ULF6 in May 2011
ISS Debris	ISS	ISS Vacuum Cleaner bag dust	Surface	Vacuum bag retains particles >6 μm ; HEPA rated filter retains particles >0.3 μm	1 day	International Space Station vacuum cleaner	Expedition 31; returned aboard Soyuz flight 29S in July 2012
JPL-SAF Debris	JPL – SAF Cleanroom Class 10K	Vacuum cleaner bag dust	Surface	HEPA rated filter retains 99.7% particles >0.3 μm	70 days	Nilfisk GM80, 81620000	Used for robotic missions
JPL-103 Debris	JPL – 103 Cleanroom Class 1K	Vacuum cleaner bag dust	Surface	HEPA rated filter retains 99.7% particles >0.3 μm	>180 days	Nilfisk GM80, 81620000	Sub-assembly of robotic missions

Checinska, A., A. J. Probst, P. Vaishampayan, J. R. White, D. Kumar, V.G. Stepanov, G.E. Fox, H.R. Nilsson, D.L. Pierson, J. Perry, K. Venkateswaran. 2015. Microbiomes of the dust particles collected from the International Space Station and Spacecraft Assembly Facilities. Microbiome 3(1):50.

Bacterial Bioburden by Various Methods



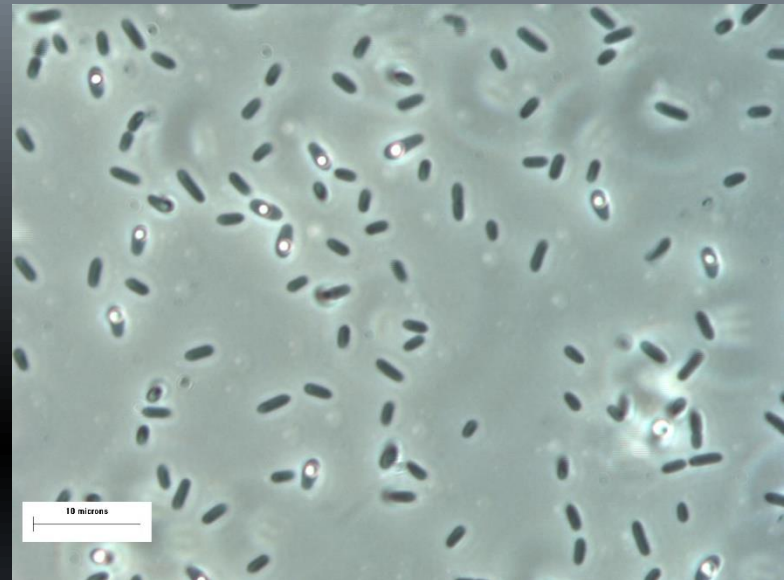
Solibacillus kalamii ISSFR-015^T

Source: ISS HEPA filter

Characteristics:

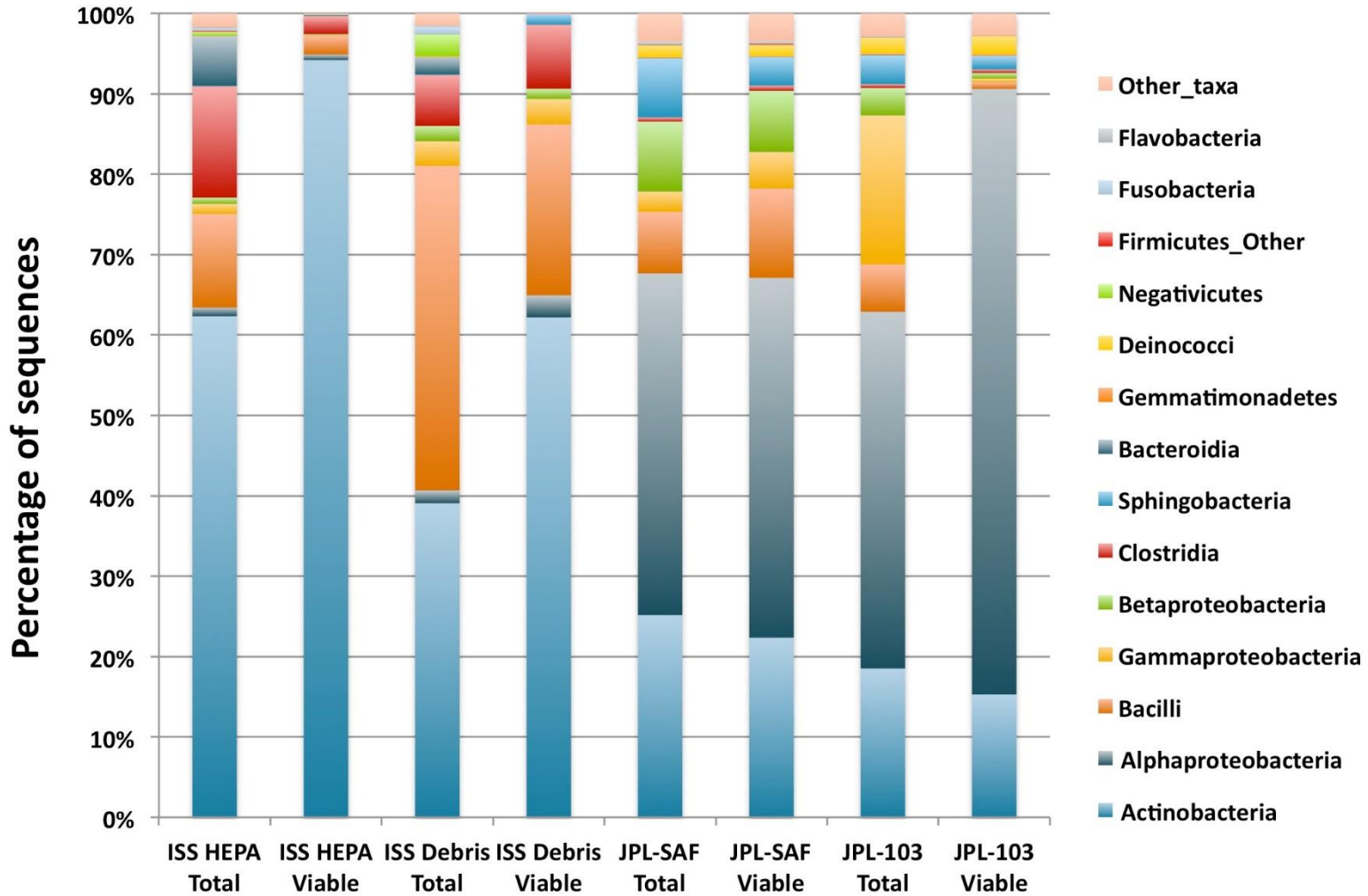
- Rod, spore-former
- A4α_L-Lys-D-Glu peptidoglycan
- MK-6, MK-7 and MK-8 isoprenoid quinones
- polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, and one unknown phospholipid

Name: ka.lam.i.i., N.L. gen. n. kalamii referring to Abdul Kalam, a well-known scientist who advanced space research in India

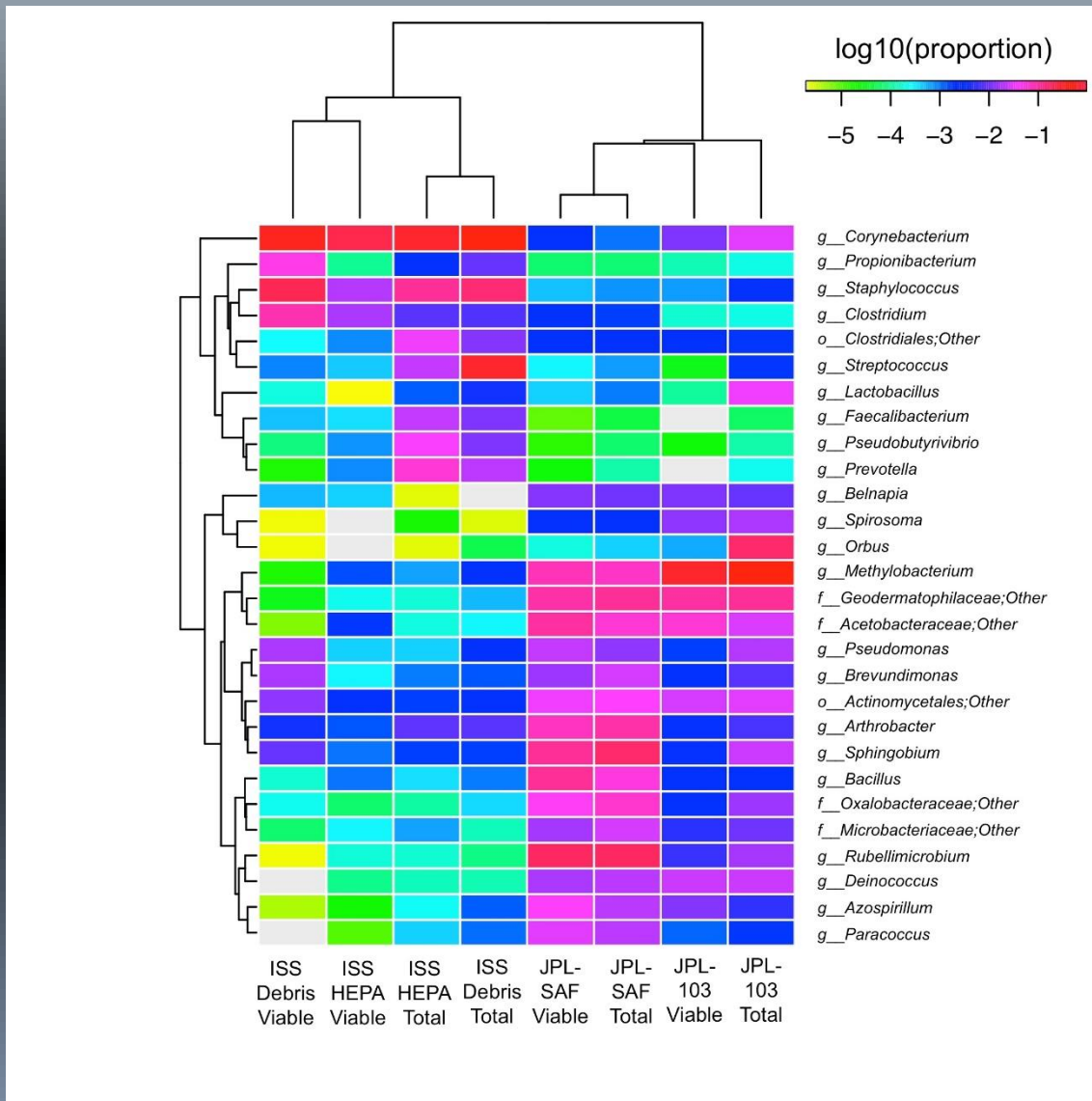


Checinska, A., R. M. Kumar, D. Pal, S. Mayilraj, K. Venkateswaran. 2016. *Solibacillus kalamii* sp. nov., isolated from a high-efficiency particulate arrestance (HEPA) filter element used in the International Space Station. Submitted to IJSEM for a review.

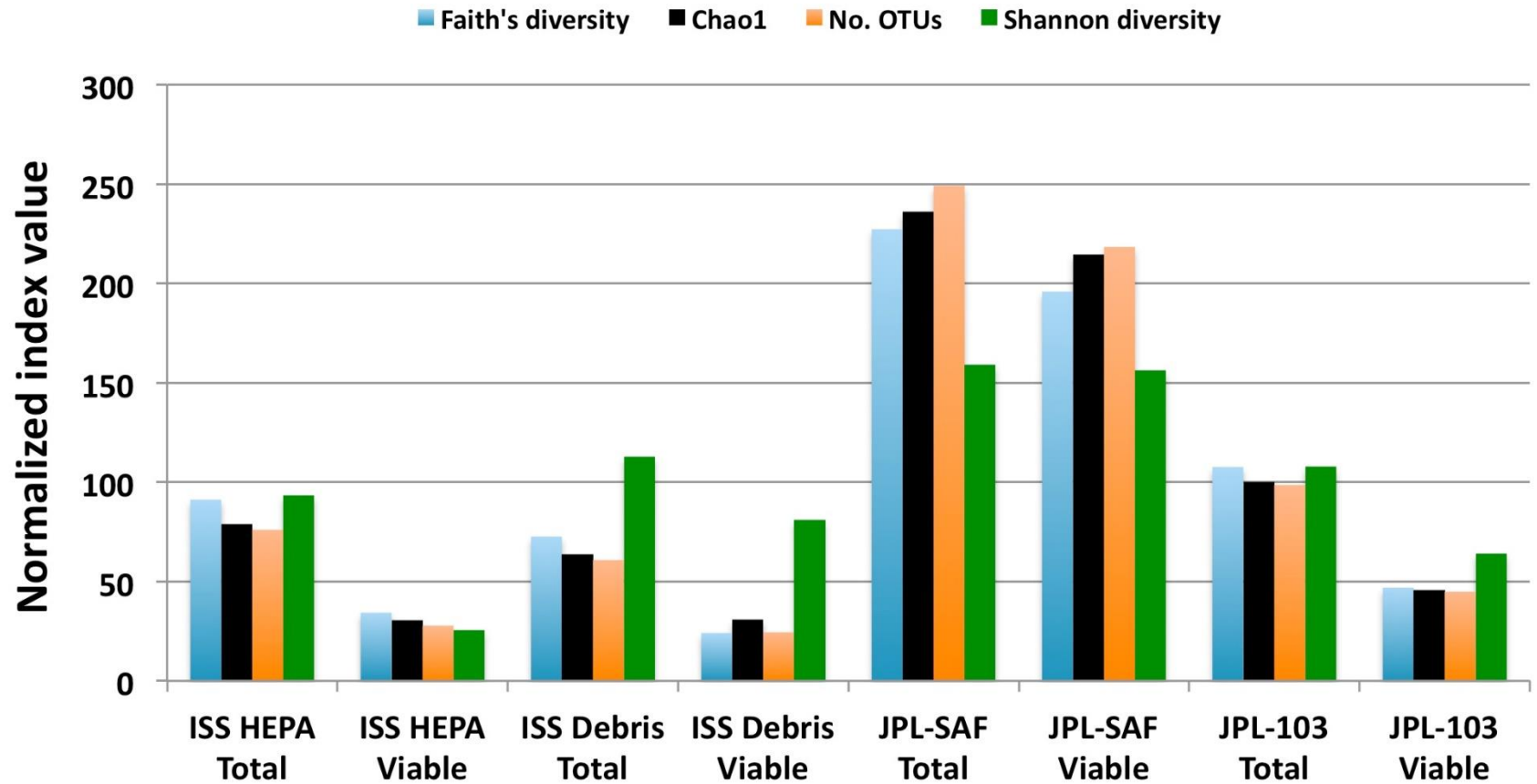
Bacterial Community Composition



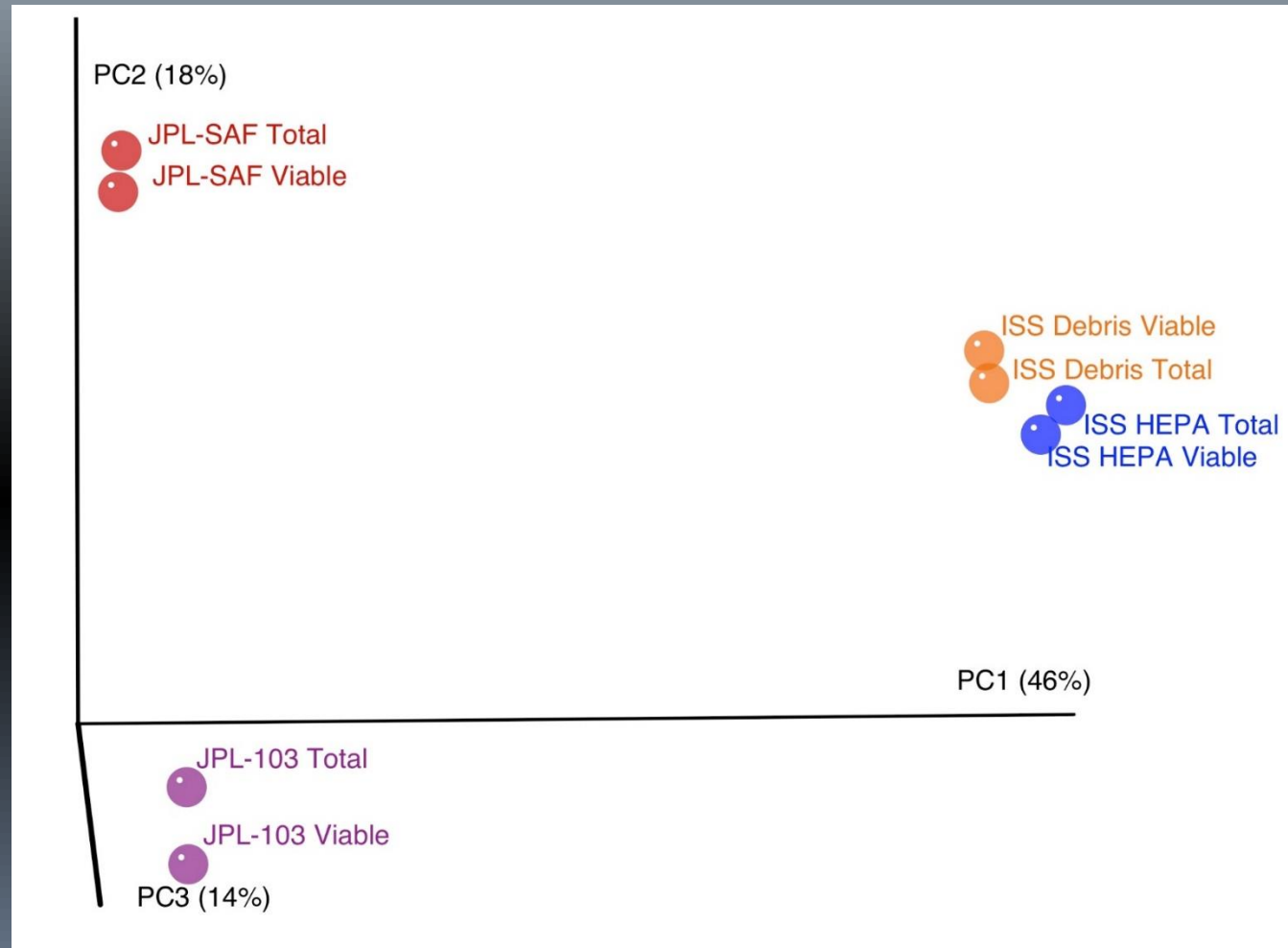
Significant Operational Taxonomic Units (OTUs)



Species Diversity



Differential Bacterial Composition of the Clean Room and the ISS Samples



Conclusions

- First study to utilize next-generation sequencing (state-of-the-art methodology) on the samples from the International Space Station
- Bacterial diversity based on the sequencing is much more diversified from culture plates.
- The composition of the ISS samples from the HEPA filter (air) and vacuum cleaner (debris) was different but still more similar to each other than to clean room samples
- Propidium monoazide treatment distinguished between dead and viable bacterial species.

Future Goals

- Environmental monitoring using rapid microbial detection and identification assay development
- Current results help develop more advanced mitigation strategies for prolonged space travel (e.g. mission to Mars)

Acknowledgments

Jet Propulsion Laboratory (Pasadena, CA, USA):

- Dr. Kasthuri Venkateswaran (Senior Research Scientist, Principal Investigator)
- Dr. Parag Vaishampayan (Scientist)
- Dr. Melissa Jones (Assistance Section Manager)

Funding:

Space Biology NNH12ZTT001N grant no. 19-12829-26 under Task Order NNN13D111T

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- Dr. Henrik Nilsson, University of Gothenburg, Sweden
- Dr. Duane Pierson, Johnson Space Center, Houston, TX, USA
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